Explor Anim Med Res.
Vol. 15, Issue 2, 2025
DOI: 10.52635/eamr/15.2.180-187

ISSN 2277-470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Published under the CC BY-NC 4.0 license

Research Article

TLR-2 DEPENDENT AND ITS ASSOCIATED CYTOKINES EXPRESSION PROFILE IN PRIMO AND REVACCINATED DAIRY CATTLE IMMUNIZED WITH AN INACTIVATED FOOT-AND-MOUTH DISEASE VACCINE

Rajeev Ranjan[1*], Jitendra Kumar Biswal[1], Smrutirekha Mallick[1], Ajay Kumar Sharma[2]

Received 15 June 2024, revised 19 October 2025

ABSTRACT: The only way to control foot-and-mouth disease (FMD) is through vaccination. The purpose of this study was to determine the TLR2 dependent and its associated cytokine expression profile in cattle. 18 animals were divided into three groups: control-naive calves (06), Gr1-primo-vaccinated calves (06), and Gr2-revaccinated adult animals (06). Gr1 and Gr2 animals were vaccinated intramuscularly with 2 ml of trivalent FMDV inactivated vaccine. On the 0th, 14th, and 21st days post vaccination (DPV), whole blood (4 ml) was collected, followed by RNA extraction and cytokine estimation by RT-qPCR. The abundance of these target genes' mRNA was calibrated against a housekeeping gene (18-S) and expressed as a fold increase over the 0th DPV control. In the control, there was no significant difference in the expression of IL-6, IL-10, IL-12, IL-17, and IL-21. IL-10, IL-17, and IL-21 were significantly upregulated in Gr-1 on the 14th DPV, whereas IL-6, IL-17, and IL-21 were significantly up regulated on the 21st DPV. On the 14th DPV, significant up regulation of IL-21 was observed in Gr-2, while significant up regulation of IL-6, IL-17, and IL-21 was observed on the 21st DPV. The current research sheds new light on the differential expression of these TLR2 dependent and its associated cytokines following vaccine administration. The presence of good antibody titre could be indicated by the up regulation of IL-6, IL-17, and IL-21 following FMD vaccination. This could lead to the use of cytokine agonists as adjuvant during vaccine formulation to modulate immune response following vaccine inoculation.

Keywords*:* Cattle, cytokine expression profile, immune response, trivalent FMDV inactivated vaccine, Real Time-qPCR.

INTRODUCTION

Foot-and-mouth disease (FMD) is an acute, infectious and highly contagious viral disease of cloven footed domestic and wild species. Disease having its devastating effects on economy and livestock trade throughout the globe. Disease is caused by *Aphthovirus,* a small, non-enveloped, single stranded, positive sense RNA virus; belong to family *Picornaviridae* [1]**.** All the seven serotypes *viz.,* O, A, C, Asia 1, SAT-1, -2, -3 of FMD virus (FMDV) are serologically and immunologically different and they do not cross reacts [2]. In India, FMD outbreak is mainly occuring due to serotypes O, A and Asia 1 [3, 4] and prevalence of serotype 'O' is the most common.

In endemic setting, vaccination is the only tool for control of FMD. Protection against FMDV in susceptible population is characterised by estimation/ detection of neutralizing antibody following either through natural infection or vaccinaion [5]. FMDV induces acute humoral antibody responses in infected or vaccinated animals, which are thought to be the most important protective factor against FMDV infection. Vaccination leads to changes in gene expression, which directly or indirectly elicit the immune system by activating immune cells of body. The importance of innate immune defences mechanisms in FMDV control, particularly during early infection [6]. However, the potential role of T-cell responses against FMDV, as well

[1]*ICAR-National Institute on Foot and Mouth Disease, International Centre for Foot and Mouth Disease, Arugul, Bhubaneswar-752050, Odisha, India.*
[2]*ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India*
**Corresponding author. e-mail: drrajraj@gmail.com*

as T-cell involvement in mediating the production of neutralising antibodies, not properly understood.

Toll like recesptors (TLRs), the receptors present on cell surface or inside the cellular compartments, which drive the immune response by utilising pathogen-associated molecular patterns (PAMPs) and pathogens detected by the pathogen recognition receptor (PRR) [7]. TLR2 is cell surface receptors which can trigger immune response by detecting the viral capsid protein and glycoprotein [8]. In case of FMDV, VP 1 and VP 3 capsid protien act as TLR-2 agonist which upregulate the TLR-2 [9].

Cytokine profiling, as a measure of T-cell responses, may provide more detailed insights into FMDV defense mechanisms. Immune response and it maintenance by prduction of Th1 and Th2 cytokines [10-12]. Cytokines are proteins and release locally at immune site by immune cells in very low concentration in response to a stimulus and often undetectable in peripheral blood circulation [13] but regulate many funciton of target cells [14]. The funcitons of cytokines depends on the state of cell and its stimulus. Cytokines may upregulate or downregulate and express different immune response [15]. Measurement of expression profile of different cytokines in FMD vaccinated cattle calf can provide valuable information and which could be beneficial for fixing vaccination regime. Previously immune respone of FMD incactivated vaccine was record in guine pig [16, 17] and calf [18].

Adjuvant-containing vaccines elicit the innate immune response by presenting antigen to T lymphocytes and promoting inflammatory reactions [19] characterized by proinflammatory cytokines, which ultimately impair cattle performance by increasing basal metabolism and tissue catabolism [19]. A variety of TLR, cytokines or its agonist are currently in use or ready to use for clinical trials as an adjuvant for vaccine delivery and this may give preventive or theraeutic effects against various diseases like HIV, TB, various type of cancer [20, 21]. Previously we reported that TLR2 and TLR3 were significantly increased in primo as well as revaccinated cattle after administration of trivalent FMDV inactivated vaccine under natural condition. But, there is paucity of literature on quantification of TLR2 dependent and its associated cytokines like interleukin (IL)-6, IL-10, IL-12, IL-17 and IL-21 expression against trivalent FMDV inactivated vaccine in cattle under natural condition. Analysing the expression profile of different TLR2 dependent and its associated cytokines using real time PCR is very important and can be used to screen immune status of animals during disease development or subsequent vaccination. Therefore, present study was performed to know the expression profile of IL-6, IL-10, IL-12, IL-17 and IL-21 against trivalent FMDV inactivated vaccine in cattle using real time RT-qPCR.

MATERIALS AND METHODS

Animals, vaccination and blood collection

The present study was carried out at the experimental cattle herd maintained at longitude 29°28'N and latitude 79°39'E. Cross bred HF cattle were received trivalent FMD inactivated vaccine (Raksha ovac Trivalent) biannually. Eighteen animals (twelve naïve calves and six adult animals) from same cohort were taken. All the animals were categorized into three groups, such as, six naïve calves (control group, age 4 month), six primo vaccinate calves (Gr1, 4 months) and the other six revaccinate adult animals (Gr2, 4-5 years). The Gr 1 and 2 animals were vaccinated intramuscularly with 2 ml of trivalent FMDV inactivated vaccine. Whole blood samples (4 ml) were collected from the jugular vein into sodium heparin Vacutainer (BD Vacutainer) at 0^{th}, 14^{th} and 21^{st} days post vaccination aseptically and immediately processed for further studies.

RNA extraction

Total RNA was extracted from whole blood using RNA isolation kit (RiboPure™- Blood, Ambion) according to manufacturer's instruction from each sample. The extracted RNA was further purified and treated with DNase I using RNeasy kit and was quantified by spectrophotometer considering the OD260/OD280 ratio, which was 1.8–2.0 for all the samples. The total RNA yields in each sample were diluted to 10 ng/µl for further analysis. Total RNA preparations were stored at - 80°C till further use.

Primer sets

Sequences of reported primers of IL-6, IL-10, IL-12, IL-17, IL-21 and the 18 S (as an internal control) genes were used for quantification of different cytokines by real time RT-PCR. Details of Real-time PCR primers for above genes showed in Table 1.

Real-time RT-PCR

Real-time RT-PCR was performed using Quanti Tect SYBR Green RT-PCR Kit (Qiagen) as previously described to quantify the cytokine expression [22]. It was carried out by a standard protocol recommended by the manufacturer with 2 µl (10ng/ µl) of total RNA,

which was added to an 18 µl reaction mixture including 0.2µmol of each primer, 10 µl of 2 x RT master mixes, 0.16 µl RT enzymes and 7.44 µl of nuclease free water. Then the mixture was run in an ABI Real Time PCR machine 7500 under the following conditions: at 50ºC for 30 min as RT, at 95 ºC for 15 min as the denaturation program, followed by 40 cycles of 94ºC for 15 s, annealing at 56 ºC for 30 s, 72 ºC for 30 s and each of optimal fluorescence measurement temperature for 1 s as the amplification and quantification programs, 60-95°C with a heating rate of 0.5ºC/s as the melting curve program and finally cooling to 40ºC as the cooling program. Samples were place in duplicate.

For each cytokine gene, it was confirmed that the efficiency of amplification was similar with 18 S gene (data not shown), permitting assessment of fold change of genes with the formula $2^{-\Delta\Delta CT}$. Therefore, fold change of mRNA was determined from the threshold cycle (C_T) values normalized for 18S gene expression and then normalized to the value derived from non vaccinated animals at 0 days [23]. In last absolute values is obtained by following formula:

Fold change= $2^{-\Delta\Delta CT} = 2^{-\Delta (\Delta CT \text{ Sample} - \Delta CT \text{ Control})}$

Where, E= Efficiency= 2, if efficiency is 100%

ΔCT Sample= CT of Cytokine at (t) day CT of 18S at (t) day

ΔCT Calibrator= CT of Cytokine at (0) day- CT of 18S at (0) day

$\Delta\Delta CT = [(CT_{\text{gene of interest}} - CT_{18S})_{\text{vaccinated sample}} - (CT_{\text{gene of interest}} - CT_{18S})_{\text{control sample}})]$

Statistical analysis

The statistical significance of cytokine genes expression between blood samplings was tested by Student's two-tailed t-test, assuming equal variances. Differences between of them were considered significant if probability values of $P<0.05$ were obtained. The statistical tests were performed by using Microsoft excel software.

RESULTS AND DISCUSSION

Vaccination is the only tool for controlling FMD in endemic areas by activating immune cells. Cytokines are proteins that immune cells release locally at the immune site and regulate many functions of the target cell. There is a lack of knowledge about the differential expression of TLR 2 dependent and its associated cytokines in response to trivalent FMDV inactivated vaccine inoculation in cattle. The measurement of the expression profile of different cytokines in FMD-vaccinated cattle can provide useful information for adjusting the vaccination regime. The purpose of this study was to investigate the immune response in FMD primo and revaccinated animals, as well as the differential expression of IL-6, IL-10, IL-12, IL-17, and IL-21 in cattle using RT-qPCR after trivalent FMDV inactivated vaccine inoculation. In brief, mechanism of action of this vaccine has depicted in Fig. 1.

Blood samples from all three groups, control (naive calves-06); primo vaccinated calves-06; and revaccinated adult animals-06, were analysed for expression of IL-6, IL-10, IL-12, IL-17, and IL-21 genes by RT-qPCR at the 0th, 14th, and 21st days post-vaccination (DPV) against trivalent FMDV inactivated vaccine. The sharpness of a melt curve peak indicating specific cytokine amplification (Fig. 2). Fig. 3 depicts the differential expression of IL-6, IL-10, IL-12, IL-17, and IL-21. The mRNA abundance of these target genes was calibrated against a housekeeping gene (18 S) and expressed as a fold over expression of the cytokine genes in bovine over the 0th DPV control.

On the 0th, 14th, and 21st dpi, the differential expression of IL-6, IL-10, IL-12, IL-17, and IL-21 was neither up- nor down-regulated in the control group. On the 14th and 21st DPV, up regulation of cytokines IL-6, IL-10, IL-17, and IL-21 expression was observed in Gr 1, but cytokines IL-10, IL-17, and IL-21 were significantly up regulated on 14th DPV. Similarly, cytokines IL-6, IL-17, and IL-21 showed a

Table 1. Primer sequence and amplicon size (bp) details of real-time PCR performed for expression analysis of TLR2 dependent and its associated cytokines and internal control 18S genes of bovine.

Sl. No.	Gene	Forward Primer (5'- 3')	Reverse Primer (5'- 3')	Amp size (bp)
1.	IL-6	TCCAGAACGAGTATGAGG	CATCCGAATAGCTCTCAG	192
2.	IL-10	TGCTGGATGACTTTAAGGG	AGGGCAGAAAGCGATGACA	329
3.	IL-12	CTTTCTTCAAATGCAGCATTGG	GGGTCTGGGTGATACAACGAA	180
4.	IL-17	TGAGTCTGGTGGCTCTTGTG	GCCTTCTGGAGTTTGTGCTC	150
5.	IL-21	ACTGTGAGCGGTCAGCTTTT	TCCCTGTATTTGTGGCAGGT	190
6.	18 S	CTGAGAAGACGGTCGAACTTGACT	TCCGTTAATGATCCTTCCGCAGGT	90

significant difference on the 21st DPV. In Gr 2, similar up regulation of cytokines IL-6, IL-10, IL-17, and IL-21 expression was observed on 14th and 21st DPV, but cytokines IL-21 showed a significant difference on 14th DPV, while IL-6, IL-17, and IL-21 showed a significant difference on 21st DPV.

Fig. 3 depicts the mean expression profile of various cytokines. At 14th and 21st DPV, the cytokine IL-6 gene was up regulated in both primo and revaccinated groups, but significant difference was observed at 21st DPV in either group. At 14th and 21st DPV, the cytokine IL-10 gene was up regulated in both primo and revaccinated groups, but a significant difference was only observed in primo vaccinated groups, not in revaccinated groups. On the 14th and 21st DPV, IL-12 was neither up- nor down-regulated in either group. At 14th and 21st DPV, the cytokine IL-17 gene was up regulated in both primo and revaccinated groups, but a significant difference was observed only in the primo group and not in the revaccinated group, whereas a significant difference was observed in both groups at 21st DPV. At 14th and 21st DPV, the cytokine IL-21 gene was maximally up regulated, and a significant difference was observed in both groups.

Up regulation of IL-6, IL-10, IL-17, and IL-21 were observed in this study. IL-6, a proinflammatory or anti-

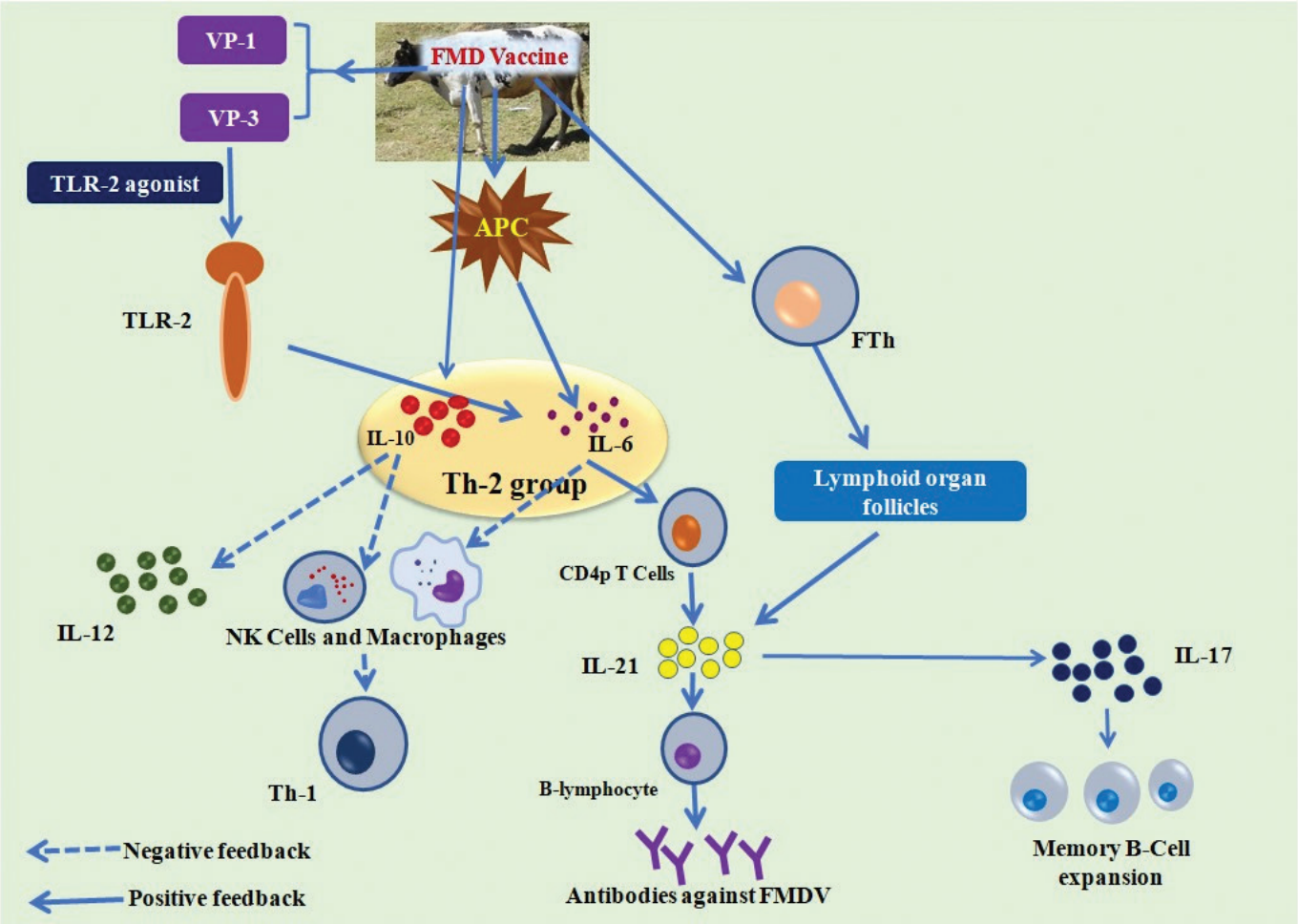

Fig. 1. Mechanism of action of TLR-2 and its dependent cytokines. [Diagrammatic representation of mechanism of action of TLR 2 dependent and its associated cytokines in cattle after intramuscular administration of 2 ml of trivalent FMD inactivated vaccine. VP1 and VP3 capsid protein of FMD virus having property of TLR 2 agonist[9] and it is responsible for upregulation of TLR-2[22]. Due to agonist activity of VP1 and VP3 capsid protein of FMD virus, up regulation of IL-6 occurred (TLR-2 dependent cytokine). CD4pT cells become active due to upregulation of IL-6 and responsible for secretion and up regulation of IL-21. Presence of IL-21 leads to responsible for antibody body production by B-lymphocyte[30]. FMD vaccine also activates follicular helper T (FTh) cells present in lymphoid organs follicles and responsible for section of IL-21[34]. IL-21 has autocrine regulatory activity on IL-17[37]. Secretion of IL-21 leads to up regulation IL-17 cytokine genes and these expressed cytokines leads to expansion of memory B-cells and ultimately leads to antibody production. IL-10 has been up regulated due to FMD vaccine[39]. IL-10 has negative feedback and inhibits the expression of IL-12 cytokine gene].

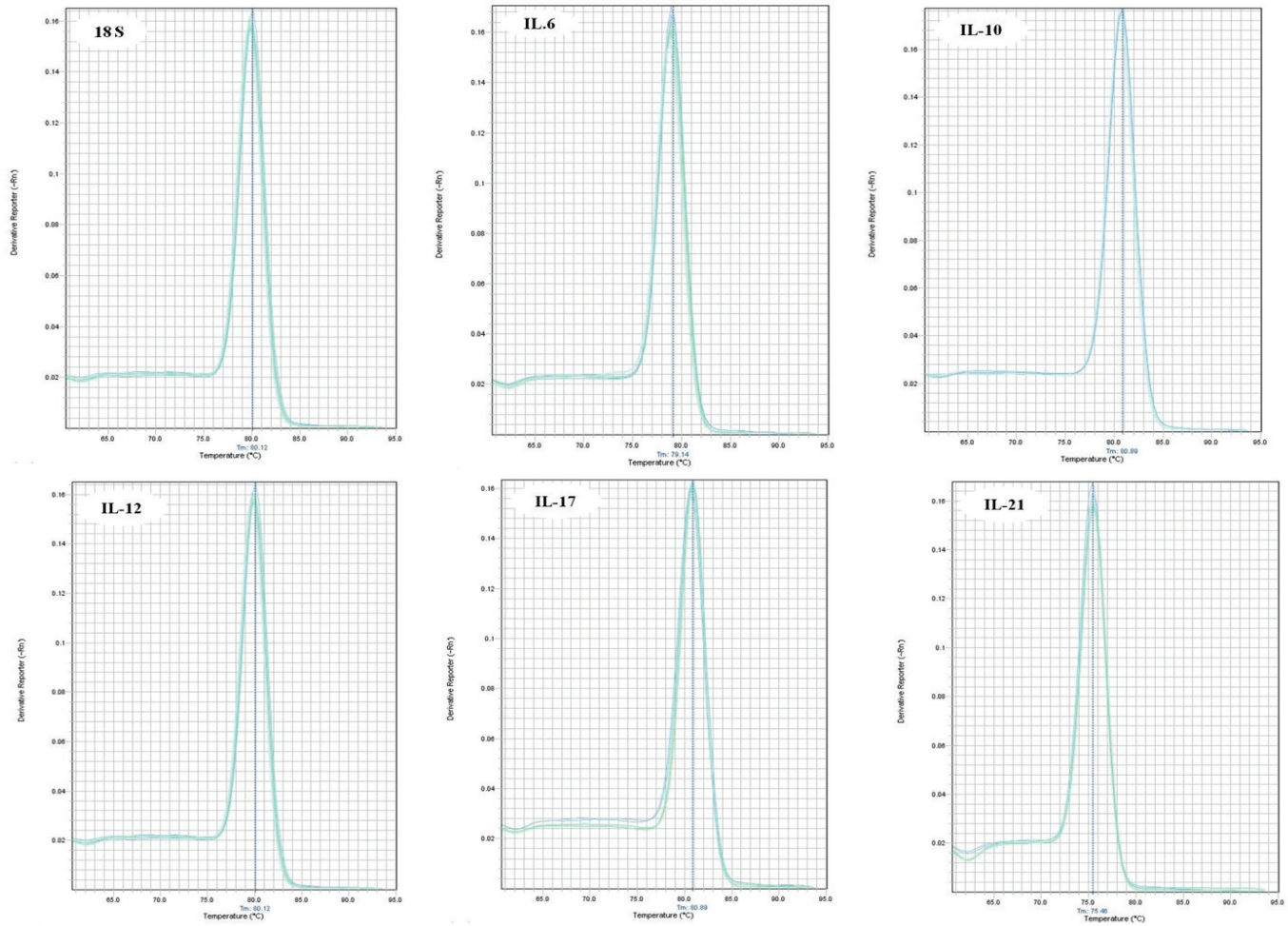

Fig. 2. Melt curve of different cytokine. [Showing sharpness of peak in a melt curve indicating specific amplification of cytokines *viz.,* 18S, IL-6, IL-10, IL-12, IL-17 and IL-21].

inflammatory cytokine produced by antigen-presenting and nonhemopoietic cells, is thought to act as a focal point for innate and adaptive immunity, mediating various aspects of B and T cell responses [24, 25], and this report discussed its impact on the humoral response [26-28]. FMDV capsid protein, VP1 and VP3, acts as a TLR2 agonist [29], which leads to TLR2 upregulation [22], and TLR 2 is ultimately responsible for the expression of TLR2 dependent cytokines IL-6. It could be the cause of the current study›s upregulation of cytokines IL-6.

IL-6 stimulated the secretion of IL-21 from CD4p T cells in response to antigen stimulation, and IL-21 then promotes antibody production by B cells [30]. It has been established that IL-21 causes antibody production by causing B lymphocytes to differentiate into plasma cells [31]. This could explain the good antibody titres against FMD vaccine in both the current (data not shown) and previous studies [18]. Vaccination may increase humoral response by activating follicular helper T (FTh) cells [32, 33]. FTh are primarily found in lymphoid organ follicles and secrete IL-21 [34]. Similarly, previous researchers have confirmed upregulation of cytokines in response to trivalent FMDV inactivated vaccine for IL-6 and IL-21 [16, 18]. The down or up-regulation of cytokine genes in Gr 2 could be due to a prior vaccine effect. Upregulation of IL-21 was observed in both groups in the current study. According to previous workers, the immune response following FMD vaccine administration may be enhanced due to upregulation of IL-6 [30, 35]. Upregulation of IL-6 and IL-21 after FMD vaccination could be an indicator of the presence of a good antibody titre against the FMD virus, and similar findings were reported in a previous report [18, 36].

Furthermore, IL 21 induces expression of IL-17 and thus it acts as an autocrine regulator of IL-17 [37]. In agreement with this, significant upregulation of IL-17 was observed in this study at 21st DPV in both the groups. IL-17 producing cells plays a vital role in host

defence mechanism by controlling innate and adaptive immunity by inducting strong memory B cell expansion in murine model against FMDV infection [38].

At 14th DPV, there was a significant increase in cytokine IL-10 expression in the primo group but not in the revaccinated group. The presence of maternal antibody, which may affect the immune response, could explain the presence of IL-10 in the primo group at 14th DPV, and a similar finding has also been reported in pigs by previous workers [17]. The expression of IL-10 in water buffalo increased after vaccination against FMD with FMD inactivated vaccine [39].

IL-6 and IL-10 are members of the Th2 cytokine group and are responsible for suppressing Th1 function. Inhibitory effect of IL-6 and IL-10 on NK cells as well as reactive oxygen species in activated macrophages [19], which may result in lower Th1 dependent cytokine production [40]. Furthermore, IL-10 inhibits the expression of IL-12, as well as costimulatory and major histocompatibility complex class II molecules [36]. In line with this, the current study found that Th2 cytokines like IL-6 and IL-10 were up regulated, while Th1 cytokines like IL-12 were neither up regulated nor down regulated.

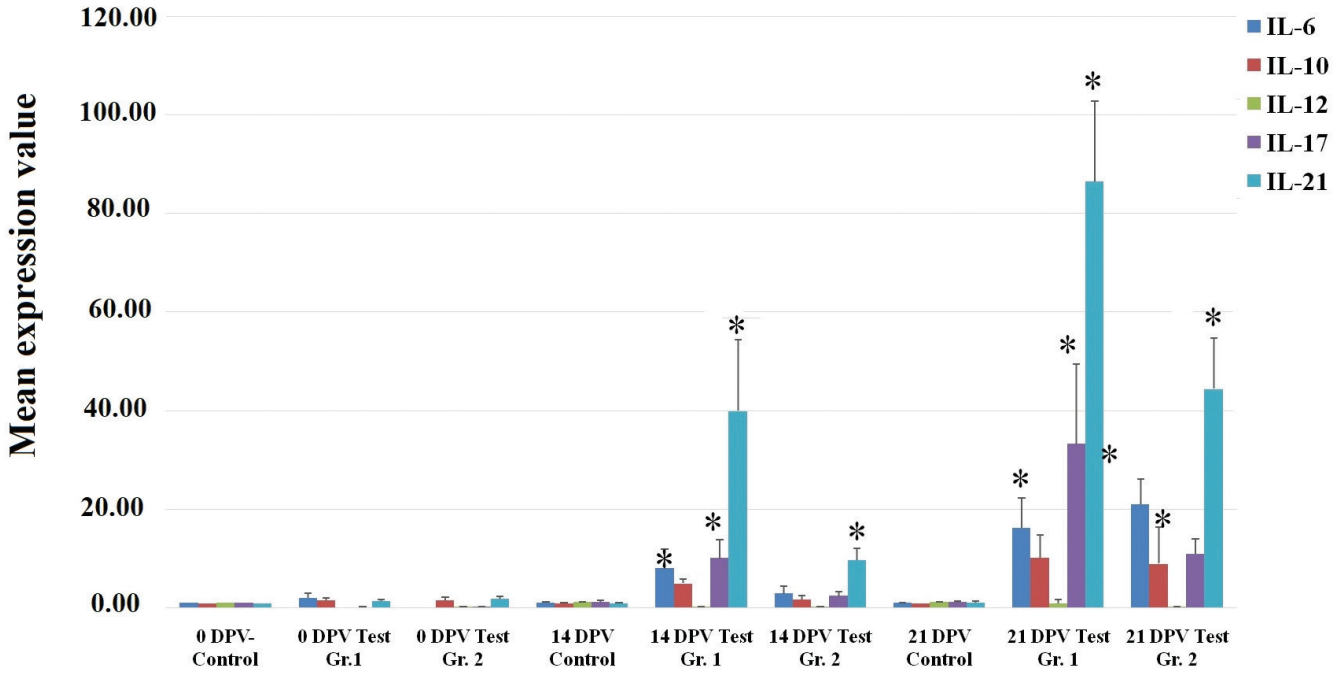

Fig. 3. Expression profile of cytokines. [Comprehensive mean expression of TLR 2 dependent and its associated cytokines genes such as IL-6, IL-10, IL-12, IL-17, and IL-21 against trivalent FMD inactivated vaccine in naive calves (control group), primo vaccinate calves (group 1), and revaccinate adult animals (group 2) at 0th, 14th and 21st days post-vaccination (DPV) was determined using RT-qPCR. Over expression of various cytokines in FMD inactivated vaccine over the 0th DPV housekeeping gene (18S)].

CONCLUSION

In conclusion, the current finding sheds new light on the differential expression of various cytokines following administration of trivalent FMD inactivated vaccine. Up regulation of IL6, IL17, and IL-21 after FMD vaccination could indicate the presence of a high antibody titre against the FMD virus. This could lead to the use of cytokines (from present study) and TLR-2 (from previous study) agonists with adjuvant during vaccine formulation to modulate immune response after trivalent FMD inactivated vaccine inoculation.

ACKNOWLEDGEMENT

ICAR-National Institute on Foot and Mouth Disease is a constituent institute of Indian Council of Agricultural Research (ICAR), New Delhi, India and ICAR has provide all financial support and necessary facilities to carry out this research work under the project number-IXX00086.

REFERENCES

1. Belsham GJ. Distinctive features of foot-and-mouth disease virus, a member of the picornavirus family; aspects of

virus protein synthesis, protein processing and structure. Proa Biophycs raolec Biol. 1993; 60(3): 241- 260.

2. Robson KJ, Harris TJ, Brown F. An assessment by competition hybridization of the sequence homology between the RNAs of the seven serotypes of FMDV. J Gen Virol. 1977; 37(2): 271- 276.

3. Biswal JK, Sanyal A, Rodriguez LL, Subramaniam S, Arzt J, *et al.* Foot-and-mouth disease: Global status and Indian perspective. Indian J Anim Sci. 2012; 82(2): 109–131.

4. Ranjan R, Kangayan M, Subramaniam S, Mohapatra JK, Biswal JK *et al*. Development and evaluation of a one-step reverse transcription-loop mediated isothermal amplification assay (RT-LAMP) for rapid detection of foot and mouth disease virus in India. Vir Dis. 2014; 25(3): 358-364.

5. Doel TR. Natural and vaccine induced immunity to FMD. CTMI. 2005; 288: 103–131.

6. Summerfield A, Guzylack-Piriou L, Harwood L, McCullough KC. Innate immune responses against foot-and-mouth disease virus: current understanding and future directions. Vet Immunol Immunopathol. 2009; 128(1-3): 205-210.

7. Takeda K, Akira S. Toll-like receptors in the innate immunity. Int Immunol. 2005; 17: 1–4.

8. Carty M, Guy C, Bowie AG. Detection of viral infections by innate immunity. Biochem Pharmacol. 2021; 183: 114316.

9. Mei LS, Yu-Ching C. Tlr-2 agonists and methods of use thereof. United States Patent, 2014; Patent No.: US 8,795,678 B2.

10. Jankovic D, Liu Z, Gause WC. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. Trends Immunol. 2001; 22(8): 450–457.

11. Maldonado-Lopez R, Moser M. Dendritic cell subsets and the regulation of Th1/Th2 responses. Semin Immunol. 2001; 13(5): 275–282.

12. Saito S, Nakashima A, Shima T, Ito M. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol. 2010; 63(6): 601-610.

13. Oppenheim JJ, Feldmann M. Introduction to the role of cytokines in innate host defense and adaptive immunity. Cellular Immunol. 2000; New York: Oxford University Press; 2000.

14. Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation. 2005; 12(5): 255-269.

15. Nadeem I, Khalid H, Samina T. Cytokines. Int J Pathol. 2004; 2(1): 47-58.

16. Sedeh FM, Khorasani A, Shafaee K, Fatolahi H, Arbabi K. Preparation of FMD type A87/IRN inactivated vaccine by gamma irradiation and the immune response on guinea pig. Indian J Microbiol. 2008; 48(3): 326-330.

17. Pasandideh R, Nassiri MR, Raouf Delgosha M, Aslaminejad AA,Tahmoorespur M *et al*. Evaluation of cytokine mRNA expression in vaccinated guinea pigs with foot-and-mouth disease type O inactivated vaccine. Arch Razi Inst. 2016; 71(1): 15-19.

18. Gowane GR, Sharma AK, Sankar M, Narayana K, Bisht P *et al*. The expression of IL6 and 21 in crossbred calves upregulated by inactivated trivalent FMD vaccine. Ani Biotech. 2014; 25 (2): 108-118.

19. Tizard IR. Veterinary Immunology, 10th edn. 2018; Elsevier, St. Louis, MO, USA, 552.

20. Moyer TJ, Zmolek AC, Irvine DJ. Beyond antigens and adjuvants: formulating future vaccines. J Clin Invest. 2016; 126(3): 799–808.

21. Ho NI, In't Veld LGMH, Raaijmakers TK, Adema GJ. Adjuvants enhancing cross-presentation by dendritic cells: the key to more effective vaccines? Front Immunol. 2018; 9: 2874.

22. Ranjan R, Biswal JK, Sharma AK, Misri J, Pattnaik B. Profiling of bovine Toll Like Receptors (TLRs) in Foot and Mouth Disease (FMD) vaccinated cattle. Indian J Anim Sci. 2016; 86(4): 367–371.

23. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C_T method. Nature Prot. 2008; 3: 1101-1108.

24. Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. J Immunol. 2005; 175: 3463–3468.

25. Kato CD, Matovu E, Mugasa CM, Nanteza A, Alibu VP. The role of cytokines in the pathogenesis and staging of *Trypanosoma brucei rhodesiense* sleeping sickness. Allergy Asthma Clin Immunol. 2016; 12: 1–10.

26. Diehl S, Rincon M. The two faces of IL-6 on Th1=Th2 differentiation. Mol Immunol. 2002; 39: 531–536.

27. Kopf M, Herren S, Wiles MV, Pepys MB, Kosco-Vilbois MH. Interleukin 6 influences germinal center

development and antibody production via a contribution of C3 complement component. J Exp Med. 1998; 88(10): 1895–1906.

28. Yang Y, Ochando J, Yopp A, *et al*. IL-6 plays a unique role in initiating c-Maf expression during early stage of CD4 T cell activation. J Immunol. 2005; 174: 2720-2729.

29. Liang SM, Chang Yu-Ching. Tlr-2 agonists and methods of use thereof. United States patent ; Patent No.: US 8,795,678 B2.

30. Dienz O, Eaton SM, Bond JP, Neveu W, Moquin D *et al*. The induction of antibody production by IL-6 is indirectly mediated by IL-21 produced by CD4+ T cells. J Exp Med. 2009; 206(1): 69-78.

31. Ettinger R, Sims GP, Fairhurst AM, Robbins R, da Silva YS *et al*. IL-21 induces differentiation of human naïve and memory B cells into antibody-secreting plasma cells. J Immunol. 2005; 175: 7867–7879.

32. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F *et al*. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J Exp Med. 2000; 192(11): 1545–1552.

33. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P *et al*. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med. 2000; 192(11): 1553-1562.

34. Moser M, Leo O. Key concepts in immunology. Vaccine. 2010; 28(3): C2–C13.

35. Yang L, Liu Z, Li J, He K, Kong L *et al.* Association of the expression of Th cytokines with peripheral CD4 and CD8 lymphocyte subsets after vaccination with FMD vaccine in Holstein young sires. Res Vet Sci. 2018; 119: 79-84.

36. Abbas A, Lichtman A, Pillai S. Cellular and Molecular Immunology. 9th edn. Elsevier: Philadelphia, PA, USA, 2018; 608.

37. Wu W, Zeng Y, Lin J, Chen T, Xun Z *et al.* IL-17 and IL-21 polymorphisms in relation to HBV related hepatocellular carcinoma in Chinese Han population. Infect Genet Evol. 2021; 87: 104638.

38. Lee MJ, Jo H, Shin SH, Kim SM, Kim B *et al.* Mincle and STING-stimulating adjuvants elicit robust cellular immunity and drive long-lasting memory responses in a foot-and-mouth disease vaccine. Fron Immunol. 2019; 10: 2509.

39. Mingala CN, Konnai S, Venturina FA, Onuma M, Ohashi K. Quantification of water buffalo (*Bubalus bubalis*) cytokine expression in response to inactivated foot-and-mouth disease (FMD) vaccine. Res Vet Sci. 2009; 87(2): 213-217.

40. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y *et al.* Interleukin-10 and related cytokines and receptors. Annu Rev Immunol. 2004; 22: 929-979.

Cite this article as: Rajeev Ranjan, Biswal JK, Mallick S, Sharma AK. TLR-2 dependent and its associated cytokines expression profile in primo and revaccinated dairy cattle immunized with an inactivated foot-and-mouth disease vaccine. Explor Anim Med Res. 2025; 15(2), DOI: 10.52635/eamr/15.2.180-187.